

## Spinal cord noradrenergic neurons are activated in hypotension

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Although norepinephrine-containing nerve terminals in the spinal cord synapse in the vicinity of sympathetic preganglionic cells, their effect on sympathetic outflow has remained unclear. Since survival during hypotension necessitates sustaining maximal sympathetic activity, we used experimental hypotension as a physiological stimulus to determine whether such activity is associated with an increase or a decrease in spinal cord norepinephrine turnover. Male Sprague-Dawley rats (500 g) were anesthetized with chloralose and urethane and their left carotid arteries were cannulated for blood pressure measurements and blood removal. Control animals remained normotensive during the 1-h study period, hypotensive animals were bled to a 50 mm Hg systolic pressure. Catecholamine release, as indicated by methoxyhydroxyphenylethyleneglycol sulfate (MHPG-SO<sub>4</sub>) concentrations, was greater in spinal cords of hypotensive rats than in normotensive controls. Apparent catecholamine synthesis also increased. Norepinephrine concentrations did not change even though those of MHPG-SO<sub>4</sub> doubled and the accumulation of dihydroxyphenylalanine (in other animals pretreated with NSD 1015) also doubled. These studies show that catecholamine-containing neurons in the spinal cord are stimulated in hypotension, and suggest that they may function physiologically to increase sympathetic outflow and thus blood pressure.

Norepinephrine's effects on sympathetic outflow depend upon the locus at which the catecholamine is released. In the posterior hypothalamus, norepinephrine release apparently increases sympathetic outflow and blood pressure (BP)<sup>21,22</sup>, while in the anterior hypothalamus and brainstem, it has the opposite effect<sup>19,20,24</sup>. The autonomic effects of the norepinephrine liberated from the descending neurons that terminate in the spinal cord remain controversial.

Using histochemical fluorescence microscopy, Carlsson et al.<sup>1</sup> and Fuxe<sup>13</sup> demonstrated that catecholamine-containing neurons terminate within the intermediolateral cell column of the spinal cord, in the vicinity of the cell bodies of sympathetic preganglionic neurons. Some authors have associated norepinephrine release from such neurons with sympathetic inhibition<sup>6,7,9,12,14,22,24</sup> while others have concluded that this release is associated with an increase in sympathetic activity<sup>14,16-21,25</sup>. For example, Chalmers and Wurtman found that [<sup>3</sup>H]norepineph-

rine turnover was accelerated in spinal cords of rabbits subjected to sinoatrial denervation<sup>2</sup>; Chalmers and Reid observed that the BP increase caused by this denervation could be blocked by intracisternal pretreatment with 6-hydroxydopamine<sup>3</sup>; and Taylor found that electrical stimulation of the cat's cervical spinal cord increased BP and that the BP increase could be blocked by giving an  $\alpha$  receptor blocker, phentolamine<sup>25</sup>.

During hemorrhagic shock, sympathetic outflow must be maximal to facilitate survival<sup>4</sup>. Sympathoadrenal catecholamine release is needed to constrict the blood vessels and thereby to increase the perfusion pressure of such vital organs as heart and brain<sup>4</sup>. We have thus examined an index of catecholamine synthesis (dihydroxyphenylalanine, or DOPA accumulation after decarboxylase inhibition), and another of norepinephrine release (methoxyhydroxyphenylethyleneglycol sulfate or MHPG-SO<sub>4</sub> levels) in spinal cords of rats subjected to hemorrhagic shock

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(or maintained at a normal BP within the study period). Our data show that hemorrhagic shock stimulates both spinal cord catecholamine synthesis and norepinephrine release.

Male Sprague-Dawley rats (500 g, retired Charles River breeders) were weighed and anesthetized with chloralose and urethane (50 and 500 mg/kg, respectively, i.p.). Tracheostomies were performed, and their left carotid arteries were cannulated with polyethylene tubing (PE 240 and 50, Clay Adams). BP was continuously recorded using a Grass model 70 polygraph and Statham transducers. To induce hypotension, animals were bled from the cannula every 5 min in 1-ml increments until systolic BP was half the starting value and additional blood was removed as necessary to maintain that pressure for 1 h. Control animals were anesthetized and cannulated, and their BPs were measured and maintained at initial values during the study period<sup>5</sup>. BPs in the control animals averaged 108 mm Hg throughout the study period; hypotensive BPs averaged 56 mmHg. After 1 h, both groups of animals were decapitated and their brains and spinal cords removed, frozen on dry ice, and stored at -70 °C.

Norepinephrine and epinephrine release (which could not be distinguished in our study) was estimated from tissue concentrations of their metabolite MHPG-SO<sub>4</sub> (refs. 8, 17), using the method of Meek and Neff<sup>16</sup> (i.e., it was isolated using a Sephadex column and then reacted with ethylenediamine to form a fluorescent derivative measured with a Beckman fluorimeter).

Rates of catecholamine synthesis were estimated by measuring the accumulation of DOPA in tissues from hypotensive and normotensive animals treated with NSD-1015 (100 mg/kg i.p., Sigma)<sup>15</sup>. NSD-1015 inhibits DOPA decarboxylase, the enzyme that converts DOPA to catecholamines; hence, the accumulation of DOPA after its administration provides an estimate of tyrosine's hydroxylation, the rate-limiting step in catecholamine synthesis<sup>15</sup>. In two sets of experiments, NSD 1015 was injected 60 min before sacrificing (1 h of hypotension; 1 h of DOPA accumulation) and in two other experiments 20 min before sacrificing (1 h of hypotension, 20 min of DOPA accumulation). The longer (1 h) time period was used to ensure the uptake of NSD 1015 into the brains of the hypotensive group (i.e., hypotension might have

been expected to slow absorption from the peritoneal space, as well as distribution to the brain).

DOPA was isolated using a Dowex column, and assayed by high-performance liquid chromatography (HPLC) using a phosphate buffer, pH 4.3, sodium octylsulfate (70 mM) and electrochemical detection.  $\alpha$ -Methyl DOPA was used as an internal standard.

Catecholamines were isolated on alumina columns and assayed by HPLC using a phosphate buffer (pH 4.25) with 0.1 mM sodium octyl sulfate, a 5- $\mu$ m ion-pairing column and electrochemical detection<sup>11</sup>. Recoveries, estimated for each sample by inclusion of dihydroxybenzylamine acid as an internal standard, averaged 81%.

Three lines of evidence indicated that spinal cord noradrenergic neurons were activated by hemorrhagic hypotension. First, catecholamine turnover, as estimated by MHPG-SO<sub>4</sub> concentrations, doubled in spinal cords of hypotensive rats compared with that in normotensive controls ( $P < 0.05$ , Table I). Second, spinal cord catecholamine concentrations did not change in the hypotensive animals (Table 1), indicating that catecholamine synthesis must have kept pace with release: i.e., catecholamine stores remained stable even as MHPG-SO<sub>4</sub> levels doubled. Third, the accumulation of DOPA in spinal cords of animals pretreated with NSD 1015 and kept hypotensive for one hour, was threefold that in cords of normotensive controls (Table I,  $P < 0.001$ ), also suggesting an acceleration of catecholamine synthesis. No significant difference in DOPA accumulation was observed when the decarboxylase inhibitor was injected during the hypotensive period and the rats were sacrificed 20 min later ( $27 \pm 6$  and  $22 \pm 3$  ng/g DOPA were found in normotensive and hypotensive animals, respectively).

Hypotension increased several indices of catecholamine synthesis and release in rat spinal cords. Concentrations of MHPG-SO<sub>4</sub>, a major metabolite of both epinephrine and norepinephrine in the central nervous system, increased when hypotensive animals were compared to normotensive controls (Table I). It is unlikely that these increases resulted from impaired MHPG-SO<sub>4</sub> transport across the brain-blood or spinal cord-blood barrier: doses of phenoxybenzamine likely to decrease BP (20 mg/kg, i.p.) have been shown to have no effect on MHPG-SO<sub>4</sub> transport from the brain<sup>17</sup>. The fact that catecholamine

TABLE I

Concentration of MHPG-SO<sub>4</sub>, catecholamines and DOPA in spinal cords from hypotensive and normotensive rats (ng/g)

Groups of 6 rats for catecholamines and 9 for MHPG-SO<sub>4</sub> were anesthetized and cannulated and then either subjected to hemorrhagic shock, or kept normotensive for 1 h. For determination of DOPA, groups of 5 animals were pretreated with 100 mg/kg NSD 1015 and were either subjected to 1 h of hemorrhagic hypotension or remained normotensive.

	Normotensive	Hypotensive
DOPA	94 ± 25	323 ± 32**
Norepinephrine	100 ± 31	105 ± 18
Epinephrine	1.4 ± 0.1	0.9 ± 0.4
MHPG-SO <sub>4</sub>	334 ± 61	696 ± 184*

\*  $P < 0.05$ , \*\*  $P < 0.001$  when data from hypotensive animals are compared with those from controls using one way analysis of variance; data represent means ± S.E.M.

concentrations did not decrease, even as those of MHPG-SO<sub>4</sub> increased (Table I), indicates that catecholamine synthesis, as well as release, increased during the hypotensive period.

Concentrations of DOPA, the product of the rate-limiting step in catecholamine synthesis, were also greater, 1 h after decarboxylase inhibition, in spinal cords of hypotensive rats than in those of normotensive controls, further suggesting an increase in catecholamine synthesis (Table I). DOPA was elevated only in the experiments where NSD 1015 was administered just prior to the 1-h hypotensive period. When the decarboxylase inhibitor was administered 40 min into the hypotensive period and DOPA allowed to accumulate for only 20 min, no difference was observed between the two groups. The differences in these experiments may reflect differences in the absorption and distribution of NSD 1015 between normotensive and hypotensive rats: during hypotension, blood is shunted away from the splanchnic circulation, thus likely impeding the absorption of NSD 1015. Moreover, cardiac output falls during hypotension, thereby reducing the drug's delivery to the

brain. Alternatively, it is possible that catecholamine-containing neurons in the spinal cord participate principally in the initial or early compensatory phase of hemorrhagic shock, such that differences between hypotensive and normotensive animals were not observed when the decarboxylase inhibitor was administered later into the hypotensive period (i.e., 20 min before the animals were killed). It is unlikely that the 1-h DOPA increase reflected non-linear accumulation: the rates at which DOPA accumulated (per min) were similar in control animals whether DOPA was allowed to accumulate for 20 or 60 min (1.4 vs 1.6 ng/g/min).

The ability of hypotension to increase catecholamine synthesis and release in the spinal cord is of special interest because catecholamine-containing neurons are known to terminate near the cell bodies of preganglionic sympathetic neurons<sup>1,13</sup> and because electrical stimulation of these neurons has been shown to influence sympathetic tone<sup>6,9,14,22</sup>. Our data show that the activity (i.e., as reflected in neurotransmitter synthesis and release) of these catecholamine-containing nerve terminals in the spinal cord is increased in animals subjected to 1 h of hypotension. Since hypotension is associated with a major increase in sympathetic outflow, our data suggest that some of the catecholamine-containing cord neurons function physiologically to increase sympathetic tone. They could do this directly, if norepinephrine is excitatory to preganglionic sympathetic neurons, or indirectly if norepinephrine inhibits an inhibitory interneuron<sup>10</sup>.

In summary, hemorrhagic shock increases catecholamine synthesis and release within the rat's spinal cord. These catecholamine-containing neurons may thus function to increase sympathetic tone.

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